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Original Article

Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil

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ABSTRACT

Citral is a typical essential oil used in the food, cosmetic, and drug industries and has shown antimicrobial activity against microorganisms. Citral is unstable and hydrophobic under normal storage conditions, so it can easily lose its bactericide activity. Nanoemulsion technology is an excellent way to hydrophilize, microencapsulate, and protect this compound. In our studies, we used a mixed surfactant to form citral-in-water nanoemulsions, and attempted to optimize the formula for preparing nanoemulsions. Citral-in-water nanoemulsions formed at S_o 0.4 to 0.6 and ultrasonic power of 18 W for 120 seconds resulted in a droplet size of < 100 nm for nanoemulsions. The observed antimicrobial activities were significantly affected by the formulation of the nanoemulsions. The observed relationship between the formulation and activity can lead to the rational design of nanoemulsion-based delivery systems for essential oils, based on the desired function of antimicrobials in the food, cosmetics, and agrochemical industries.

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1. Introduction

Nanoemulsions are a class of emulsions with droplet sizes from 20 nm to 100 nm [1]. Because of the small droplet size, nanoemulsions appear transparent or translucent and are more stable with respect to creaming, coalescence, flocculation, and Ostwald ripening than convention emulsions [2]. The physicochemical properties of nanoemulsions are interesting for practical applications because of the small droplet size and long-term stability. Nanoemulsions are used in agrochemicals in pesticide drug-delivery formulas [3], in cosmetics as drug carriers for personal care or skincare products [4], and in pharmaceuticals as matrices for encapsulation of the bioactive compounds, which are desirable for alcohol-free formulation [5].

The best nanoemulsion droplets in emulsions are prepared with the optimal hydrophilic-lipophilic balance (HLB) and optimal surfactant concentration [6]. The proper HLB value of the surfactants is important for the formation of the emulsion. Nanoemulsions are usually formulated to enhance stability by use of a mixed surfactant because of the broad-chain length distribution. Vitamin E-enriched nanoemulsions have been reported by adjusting the HLB values of the surfactants with Tween 20, 40, 60, 80, and 85 [7]. Nanoemulsions containing astaxanthin prepared with a mixed surfactant have a smaller droplet size and a narrower size distribution than regular emulsions [8].

The formation of nanoemulsions is controlled by the relationship between droplet disruption and droplet coalescence. The ultrasonic processor applies excellent shear force for droplet disruption, and the rate of droplet coalescence is determined by the mixed surfactant and the concentration [9]. There are two main mechanisms operating during ultrasonic emulsification [10]. First, an acoustic field produces interfacial waves to break the disperse phase into the continuous phase. Second, the formation of acoustic cavitation is used to collapse microbubbles into droplets of nanometric size by pressure fluctuations.

The surfactants used in this study—Span 85 and Brij 97—are commonly used as emulsion surfactants in a wide range of food, cosmetic, and pharmaceuticals products. Nanoemulsions offer significant potential as functional ingredients in foods, cosmetic, and pharmaceuticals products because of their effectiveness to deliver flavors and aroma with their increased surface area and as mechanisms for delivering hydrophobic biocompounds. Therefore, it would be advantageous to formulate nanoemulsions with mixed surfactants, which have become widely acceptable in formulations of these types [11]. Brij-type surfactants possess a branched hydrophilic region of three polyoxyethylene chains substituted with a sorbitan ring, and Span-type surfactants have a large head group that can potentially aggregate at the Q3 o/w interface and form a hydrophobic region.

Citral (3, 7-dimethyl-2, 6-octadienal) is a monoterpene that occurs naturally in herbs, plants, and citrus fruits [12]. Citral possesses antifungal activity and bactericidal [13], insecticidal [14], deodorant, expectorant, appetite stimulating, and spasmolytic properties; it has weak diuretic and anti-inflammatory effects [15]. However, citral is susceptible to oxidative degradation, which results in the loss of antimicrobial activity under normal storage conditions. Citral is also insoluble in water at neutral pH; therefore, nanoemulsion technology appears to be a good way to microencapsulate, solubilize, and protect this compound.

Nanoemulsion-based delivery systems with incorporated constituents significantly increasing the antimicrobial activity compared with nonencapsulated systems have promise [16–18]. The systems can increase the concentration of the bioactive compounds in areas where microorganisms are preferably located. The main objective of this study was to investigate the optimal conditions for preparing citral-in-water nanoemulsions with mixed surfactants using ultrasonic emulsification. Additionally, we evaluated the antimicrobial activity of citral nanoemulsions against bacteria.

2. Materials and methods

2.1. Materials

The citral (mixture of cis- and trans-isomers, 95% pure, of plant origin) used in this study was obtained from Merck (Darmstadt, Germany). Reagent grade Span 85 (sorbitane trioleate) and Brij 97 [polyoxyethylene (10) oleyl ether] with average HLB values of 1.8 and 12.0, respectively, were from Sigma (Deisenhofen, Germany). The ethylene glycol ($C_2H_6O_2$, M.W. = 62 g/mol), used as a cosolvent in the emulsion system, was from Merck. The water used in this study was deionized and filtered using a Milli-Q system (Millipore Corp., Molsheim, France).

2.2. Nanoemulsion preparation

Nanoemulsions consisted of citral, a mixed surfactant, deionized water, and a cosolvent. The concentration of citral was fixed in 10%, and the HLB values of the mixed surfactant varied from 2 to 12. The HLB values of the mixed surfactant were calculated as follows: $HLB_{mix} = HLB_S \cdot S\% + HLB_P \cdot P\%$, where HLB_S, HLB_P, and HLB_{mix} are the HLB values for Span 85, Brij 97, and the mixed surfactants, respectively, and S% and P % are the mass percentages of Span 85 and Brij 97 in the mixed surfactants, respectively. The HLB value of the surfactants was considered to be the algebraic average of the HLB value of the individual surfactant. The ratio of mixed surfactant to citral was expressed by the ratio S_o. The cosolvent concentration was fixed at 1%.

All emulsions were prepared in two stages. The coarse emulsion was obtained using a Polytron (PT-MR 3000, Kinematica AG, Littau, Switzerland), and then further emulsified with an ultrasonic process. Coarse emulsions containing different compositions were prepared at the highest speed for 10 minutes with a sample volume of about 30 mL each time. Ultrasonic emulsification involved use of a 20-kHz Sonicator 3000 (Misonix Inc., Farmingdale, NY, USA) with a 20-mm diameter tip horn. The tip of the horn was positioned symmetrically in the coarse emulsion, and the experiment was initiated at various preset ultrasonic nominal powers (6–51 W) for 30300 seconds and controlled by the device software. During emulsification, the difference in temperature